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Innervation of the Caudal-Fin Muscles in the Teleost Fish, Medaka (*Oryzias Latipes*)

Yuji Ishikawa[1]

Department of Anatomy, Faculty of Medicine, University of the Ryukyus, Okinawa 903-01, Japan

ABSTRACT—The peripheral pattern of the spinal nerves in the caudal fin and the spinal motoneurons that innervate the caudal-fin muscles were described in the adult medaka. The peripheral nerves were immunochemically stained by using antibodies to neurofilament proteins. To label the spinal motoneurons retrogradely, horseradish peroxidase was applied to each caudal-fin muscle. The somata of motoneurons innervating the caudal-fin muscles were distributed over the whole area in the motor column from spinal segment 25 to 28 of the ipsilateral spinal cord. Most motoneurons were small (6–15 micra in diameter), but larger ones (more than 20 micra in diameter) were sometimes found. The axon of each motoneuron, rather than entering the nearest ventral root, ran caudad in the anterior funiculus of the spinal cord for the length of several spinal segments before entering a single ventral root. The axon that starts from the anterior segment of the spinal cord entered the anterior ventral root. Each caudal-fin muscle was innervated by several ventral roots of the spinal nerves from 27 to 31B. The anterior caudal-fin muscles were innervated by the anterior spinal nerves. Thus, motoneurons that innervate caudal-fin muscles are organized somatotopically along the cranio-caudal axis of the spinal cord.

INTRODUCTION

The medaka (*Oryzias latipes*) is suitable for observing in detail the nerve pattern in whole mounts, since the tail regions are thin and almost transparent in the embryonic stages [1–3]. Moreover, some inbred strains of the medaka have become available for experimental work [4], and more than 60 mutant strains including several morphogenetic ones have been found in the medaka [5, 6]. The first transgenic fish has been produced successfully in the medaka [7]. Hence, this teleost fish affords good material for experimental studies in developmental neurobiology of vertebrates [see also 8–12].

Anatomical study is an essential step in investigating the development of the nervous system in the medaka. However, little information on the neuroanatomy of the medaka is available. The present study began as an effort to understand how the muscle nerves develop in the caudal fin. This paper describes the anatomy of the nerve-muscle system in the caudal region of the adult medaka. Special attention is focused on the innervation of one of the caudal-fin muscles, the middle interradial muscle (MIR muscle), since its development will be described in detail in forthcoming papers.

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[1] Present address: Division of Biology, National Institute of Radiological Sciences, Chiba 263, Japan.

MATERIALS AND METHODS

Materials

The d-rR strain of the medaka (*Oryzias latipes*) was given by Dr. H. Tomita (Nagoya University) in March 1986. The fish of this color mutant strain shows the wild-type morphology, except for the mutations in amounts and distribution of several kinds of pigment cells [5]. The fish have been kept in our laboratory in plastic aquaria and fed a diet of Tetra-min (Tetra, West Germany). Adult fish and young adult fish (15–30 mm in total body length) of the d-rR strain were used. At least three fish were used in each labeling experiment and in each staining (see below). Fry (7–15 mm in total

body length) were also used to show general structures of the medaka, since the gross patterns of skeleton, muscles, and peripheral nerves in the caudal region of the adult fish are almost identical, except for their sizes, to those of the fry longer than 7 mm in total body length [2].

Retrograde Labeling with HRP

Adult fish were anesthetized in 0.01–0.03% MS 222 and secured to a plastic plate with a gluey tape. The unilateral skin above the muscle to be examined was cut and the underlying muscle tissue was macerated with a sharpened steel needle. Small crystals of horseradish peroxidase (HRP, Toyobo, Osaka, Japan; Lot No. 1417) were put on the wound. The fish was put in fresh water in such a way that the head and gill regions were in the water while the wounded tail region was above the water surface to avoid dilution or leakage of the HRP. After 30 min, the fish was washed in fresh water, released into the balanced salt solution [8], and allowed to survive for 24–48 hr.

After that, the fish was anesthetized heavily and killed by decapitation. The caudal part of the body was put in a solution of 2 or 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). In the fixative, the spinal cord (including the proximal portions of the ventral roots) and the vertebral column were isolated from the other tissue. The spinal cord and the vertebral column were fixed in fresh fixative overnight at 4°C. After washings over a period of several days with phosphate-buffered saline (PBS) at 4°C, HRP was histochemically detected in whole mounts by the methods of Nordlander [13] and Hanker *et al.* [14]. The spinal cord was cleared with glycerin and observed in whole mount.

Celloidin Sections of the HRP-labeled Spinal Cord

In order to examine in detail the HRP-labeled cells in the spinal cord, the celloidin-sectioned specimens were prepared from the spinal cords which had been reacted as described above. After washing in PBS, the reacted spinal cords were fixed again in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4). They were dehydrated, embedded in celloidin, and sectioned serially (22 micra in thickness). Nissl staining was performed in alternate sections by staining with thionin.

Labeling with DiI

In some caudal-fin muscles, 1,1′-dioctadecyl-3,3,3′-3′-tetramethylindocarbocyanine perchlorate (DiI, Molecular Probes, Junction City, Oregon) was used to label retrogradely the motoneurons [15]. Young adult fish were fixed in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 4 h. After washing in PBS, a lesion was made on the muscle to be examined with a small scalpel or a sharpened steel needle, and a small particle of DiI was put on the wound. The fish was placed in a moist chamber and kept moist for 10–48 h at room temperature (28°C). After the DiI was rinsed off in PBS, the spinal cord with the vertebral column was isolated. It was mounted in PBS and examined under a fluorescent microscope equipped with a set of filters appropriate for rhodamine fluorescence microscopy.

Nerve Staining

Nerve fibers were stained in whole mounts using anti-neurofilament protein monoclonal antibodies (all neurofilament proteins, 70 K+160 K+210 K, Maruzen Oil Biochemical or Cosmo Bio Co., LTD., Tokyo) according to a previously reported method [1, 16].

Muscle Staining

The muscle fibers and myotubes were immunohistochemically stained in whole mounts using a monoclonal antibody against chicken troponin T according to the method of Ishikawa [2].

Double Staining of Muscle and Nerve

The nerve staining and the muscle staining were combined so that nerve and muscle could be visualized simultaneously in the same specimens.

The muscle fibers and myotubes were first reacted with the anti-troponin T antibody and the HRP-labeled secondary antibody in whole mounts as described above. They were stained with peroxidase reaction using 3,3′-diaminobenzidine (DAB) as a substrate. The stained specimens were washed in 0.1 M glycine-HCl buffer (pH 2.4) overnight at room temperature (28°C) to remove the anti-

troponin T antibody and the HRP-labeled antibody. The DAB reaction product (brown in color) remained on the muscle tissue. After washing in PBS, the nerve fibers of the specimens were then reacted with anti-neurofilament protein antibody and the HRP-labeled secondary antibody. They were stained with peroxidase reaction, this time, using 4-Cl-1-naphthol as a substrate to obtain a blue reaction product. The specimens were cleared with glycerin and immediately observed.

Staining of Acetylcholine Receptors

For observation of neuromuscular junctions, acetylcholine receptors (AChR) were stained in whole mounts by the indirect HRP-labeled antibody method, using erabutoxin b [17], one of the curaremimetic toxins from snake venom, and an anti-erabutoxin b polyclonal antibody [cf. 18].

Nomenclature

We followed Ishikawa [2] in the nomenclature of bones and muscles in the caudal region of the medaka.

There was a problem in numbering the spinal nerves since the two cranialmost spinal nerves did not emerge from the vertebral column but instead, emerged from the skull in the medaka (see results). In the present paper these two spinal nerves were referred to as occipito-spinal nerves according to Ray's description [19] of the cranialmost spinal nerves of a teleost fish (see refs. [20], [21] for detailed discussion on the nomenclature of the rostral spinal nerves in fish; see ref. [22] for an alternative numbering of the spinal nerves of the medaka).

RESULTS

Spinal Nerves and Caudal-Fin Muscles

Prior to describing the innervation of caudal-fin muscles, we provide brief accounts of the ana-

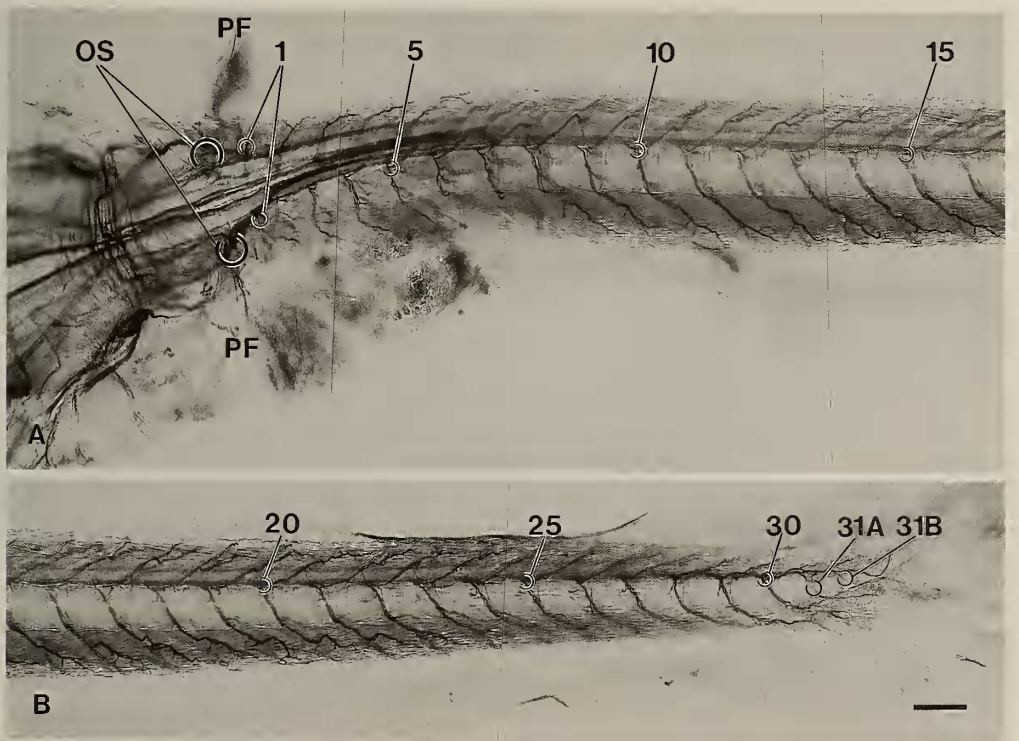


FIG. 1. Occipito-spinal nerves (OS) and spinal nerves (numbered) of a fry (A and B). The nerves were stained immunochemically using anti-neurofilament protein antibodies. The left lateral view of the whole-mount specimen is shown. The head is tilted dorsally. PF, pectoral fin. Scale bar=0.1 mm.

tomical features of the spinal nerves and caudal-fin muscles.

Two pairs of the occipito-spinal nerves and 32 pairs of the spinal nerves were usually observed in the medaka (Fig. 1). The occipito-spinal nerve 1, which is the first postvagal nerve, emerged through a foramen in the skull. The occipito-spinal nerve 2, the second postvagal nerve, emerged immediately caudal to the skull. Spinal nerve 1, the third postvagal nerve, passed through a foramen in the first vertebra. Four cranialmost nerves, namely, the occipito-spinal nerve 1, the occipito-spinal nerve 2, the spinal nerve 1, and the spinal nerve 2 formed the cervico-brachial plexus at the base of the pectoral fin. In the caudalmost region, two pairs of spinal nerves (31A and 31B) emerged through the foramina in the same caudalmost vertebra, namely the 31st vertebra.

Figure 2 shows the main branches of a typical spinal nerve in the tail region. The ventral root (VR) was larger than the dorsal root (DR), and extended through its own formen in the vertebra. Outside the vertebral column the lower part of the ventral root turned downward to form the ramus ventralis, while the upper part turned dorsad to join the spinal ganglion which formed on the dorsal root. The ramus dorsalis originated near this ganglion.

The ramus ventralis coursed ventro-caudally in the ventral region of its own segment and projected many side branches. The largest side branch projected laterally from the ramus ventralis to form a lateral branch at the level of the horizontal septum. The lateral branch ran toward the lateral surface of the segmental muscle and bifurcated dorsally and ventrally near the posterior

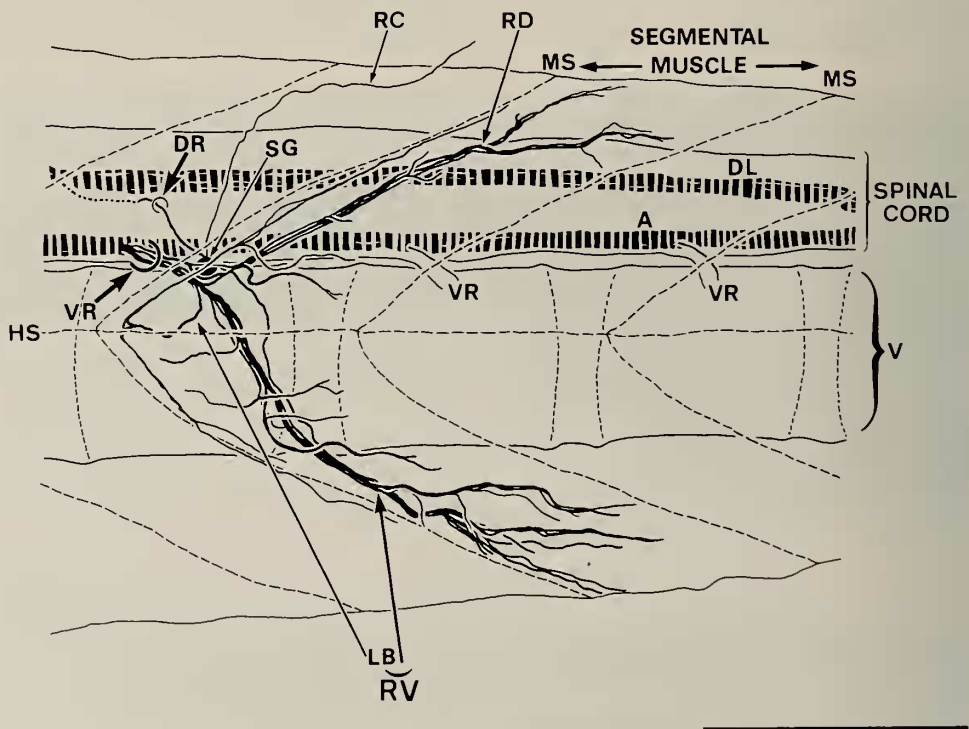


FIG. 2. Drawing of the peripheral pattern of a typical spinal nerve (spinal nerve 25) in the tail region of a fry. The nerve of the fry were stained immunochemically using anti-neurofilament protein antibodies, and the main nerve branches were traced. The left lateral view is shown. A, nerve bundles in the anterior funiculus; DL, nerve bundles in the dorsal portion of the lateral funiculus; DR, dorsal root; HS, horizontal septum; LB, lateral branch; MS, myoseptum; RC, ramus communicans; RD, ramus dorsalis; RV, ramus ventralis; SG, spinal ganglion; V, developing vertebral column, VR, ventral root. Scale bar=0.1 mm.

lateral line nerve in the horizontal septum. The bifurcated branches ran near the anterior myoseptum. The ramus dorsalis coursed dorso-caudally in the dorsal region of its own segment. It received a small branch (ramus communicans) from the ramus dorsalis of the next anterior spinal nerve.

The supporting skeleton of the caudal fin was overlaid with two groups of caudal-fin muscles, namely, the anterior caudal-fin muscles and the posterior caudal-fin muscles (Fig. 3).

In the anterior caudal-fin muscles, a pair of deep dorsal flexors (DDF) and a pair of deep ventral flexors (DVF) were present in the dorsal and ventral regions, respectively. There might be superficial dorsal flexors and superficial ventral flexors on the surfaces of these two muscles. However, it was difficult to discriminate between the caudal superficial muscles and the caudal deep muscles. Hence, in the present study, DDF and DVF are defined as containing in entirety both the deep and superficial muscle layers. A pair of the hypochordal longitudinal muscles (HLM) was situated between the two hypural plates.

In the posterior caudal-fin muscles, 13 to 15 pairs (adult fish) or 8 pairs (young adult fish) of the interradial muscles were observed beteen the fin rays. The centrally positioned muscle is called the middle interradial muscle (MIR muscle). The MIR muscle was located between the HLM and the caudalmost pit organ.

Labeling with HRP

In order to estimate the diffusion of HRP from the wound, we performed a series of experiments in which HRP activities were reacted in the whole specimens prior to the isolation of spinal cords.

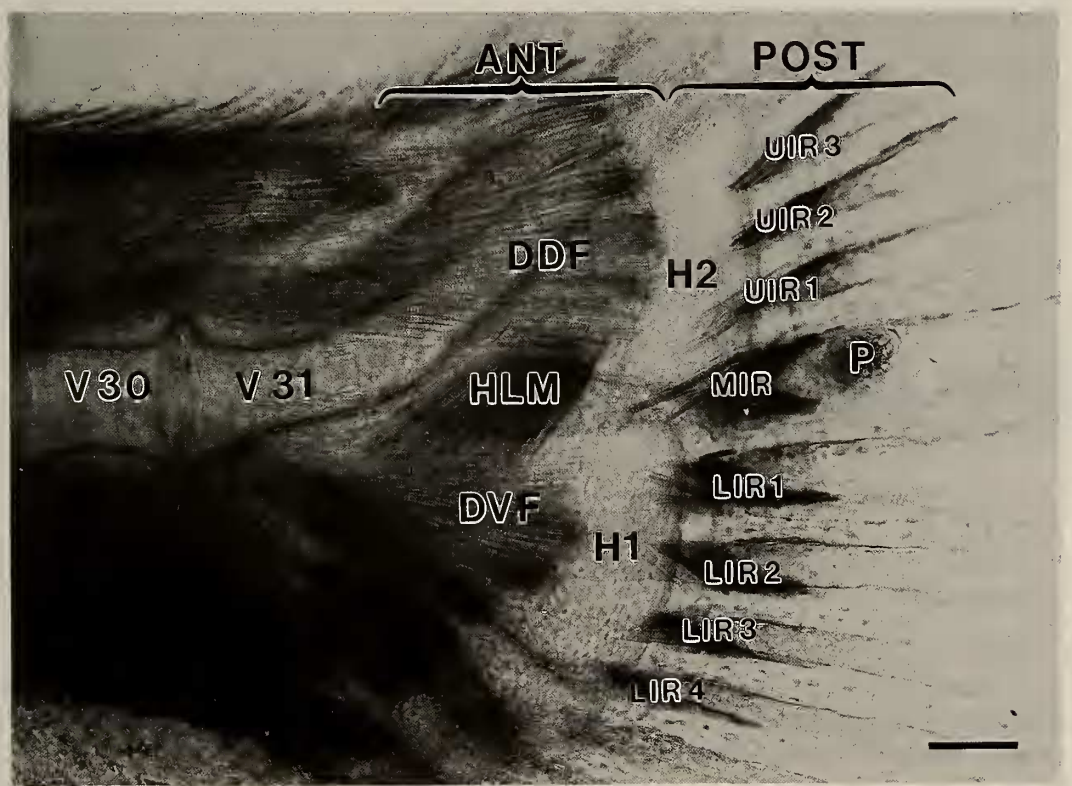


FIG. 3. Anterior (ANT) and posterior (POST) caudal-fin muscles in a young adult fish. The muscles were stained immunochemically using anti-troponin T antibody. The left lateral view of the whole-mount specimen is shown. The vertebrae (V30 and V31) and hypural plates (H1 and H2) are also shown. DDF, deep dorsal flexor; DVF, deep ventral flexor; HLM, hypochordal longitudinal muscle; LIR, lower interradial muscle; MIR, middle interradial muscle; UIR, upper interradial muscle; P, caudalmost pit organ. Scale bar=0.1 mm.

From the results, it appeared that HRP did not diffuse from the site of the application (Fig. 4). However, in the case of the interradial muscles, we could not rule out a diffusion of HRP to the same muscle in the opposite side: In some cases, the interradial muscles in the opposite side turned brown after the reaction. In such cases, there were several lightly stained neurons in the contralateral sides of the spinal cords that may have been labeled by a small amount of diffused HRP. In the present study, therefore, only darkly stained Golgi like cells were considered to be labeled motoneurons innervating the HRP-applied muscle.

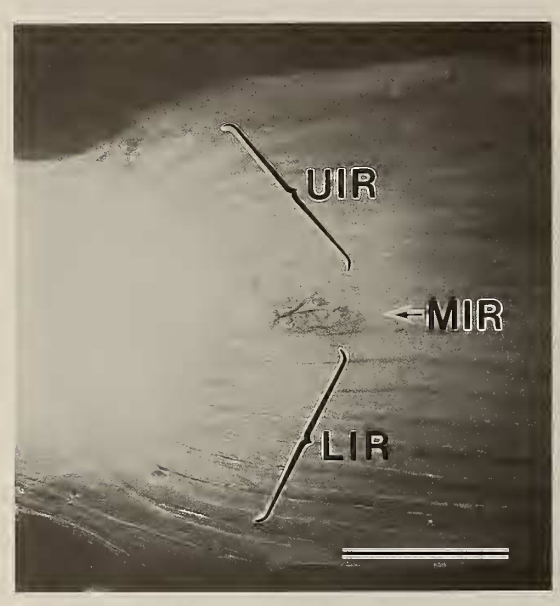


FIG. 4. HRP-positive area in the caudal fin of an adult fish after applying HRP to the MIR muscle (arrow). Note that the HRP activity is restricted in the MIR-muscle region. Left lateral view of the whole-mount specimen is shown. For abbreviations, see Figure 3. Scale bar = 1 mm.

At least three animals were used for the labeling experiments of each muscle. The results in each muscle were generally consistent in all experiments.

Labeled Motoneurons

The somata of the labeled motoneurons were either spherical or ellipsoidal and found in the ipsilateral side of the spinal cord (Fig. 5). There appeared to be two populations which were different in the soma size of the labeled motoneurons (Figs. 6B, 6C and 7), The smaller ones (small motoneurons, Fig. 6B), to which most of the labeled motoneurons belonged, were 6–15 micra in diameter, while the larger ones (large motoneurons, Fig. 6C) were few in number and were more than 20 micra in diameter. The anterior caudal-fin muscles (DDF, HLM, and DVF) were innervated by both the small and large motoneurons, whereas the posterior caudal-fin muscles (interradial muscles) were innervated only by the small motoneurons (Fig. 7).

The labeled small and large motoneurons usually had two primary dendrites, one running dorsorostrally and the other dorso-caudally; both dendrites ran in the lateral funiculus of the spinal cord, and branched along their entire courses (Figs. 5B and 6C). The cranio-caudal extension of the dendrites was large in the motoneurons filled from the anterior caudal-fin muscles and small in those filled from the posterior caudal-fin muscles.

An axon emerged from the ventral portion of the soma as a single tapering process that extended ventro-caudally and ipsilaterally in all motoneurons (Figs. 5A and 6A). Axon collaterals were sometimes observed in the initial part of the axon (Fig. 6B). The axon did not emerge immediately *via* the nearest ventral root, but ran caudad in the anterior funiculus of the spinal cord for several (2–4) segment lengths before entering a single ventral root (Fig. 6A). The axon of the motoneuron that lies in the more cranial segment of the spinal cord ran the more ventral course in the anterior funiculus of the spinal cord and emerged from the more cranial ventral root.

Location of Labeled Motoneurons in the Spinal Cord

The spinal motor column in the cross plane was revealed by plotting the locations of the somata of the HRP-labeled motoneurons in a schematic cross plane of the spinal cord (Fig. 8). The HRP-labeled motoneurons filled from each muscle were found over the whole area of the motor column: No restricted localization of any specific groups of the motoneurons was found in the motor column in

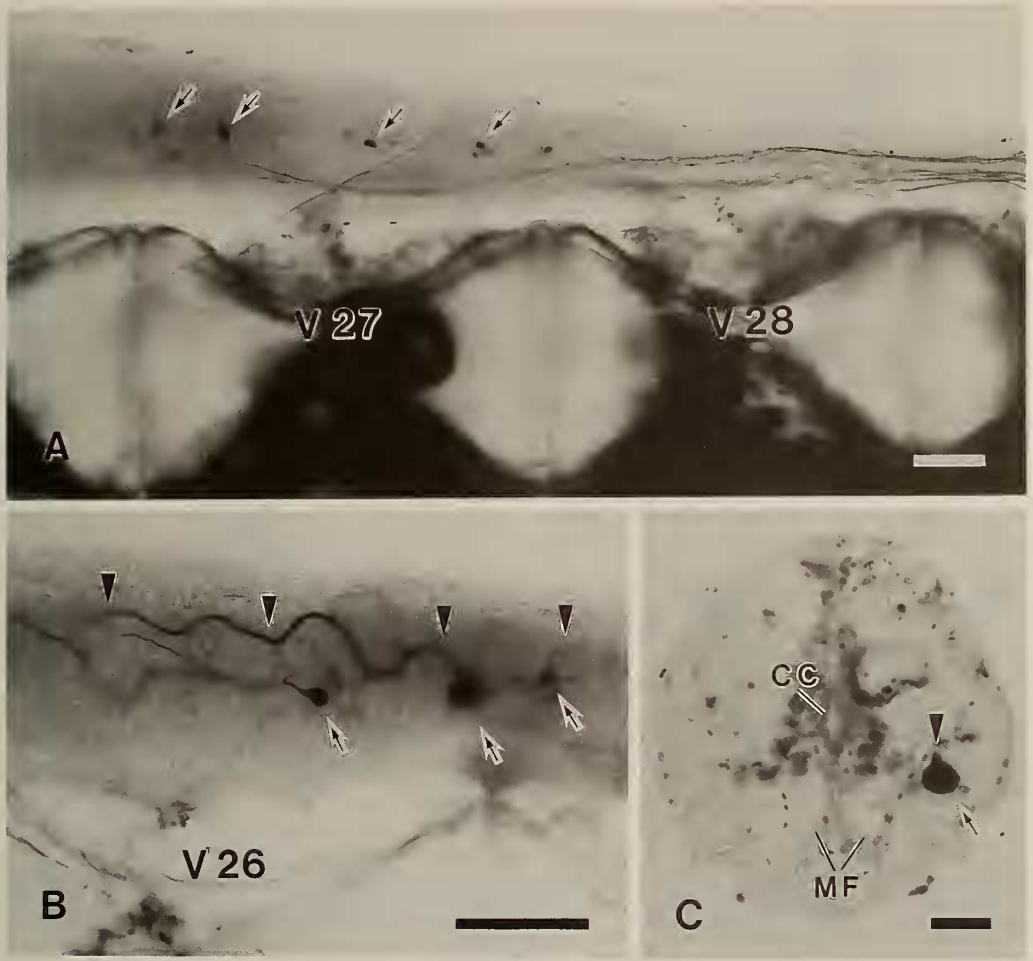


FIG. 5. HRP-labeled motoneurons (arrows) in the adult spinal cords, after applying HRP to the MIR muscle (A) and the HLM (B and C). The left lateral views of whole mount specimens (A and B), and the cross section (22 micra in thickness) of the spinal cord embedded celloidin (C) are shown. The cross section is Nissl-stained to show the gray matter in the spinal cord. The dendrites are indicated by arrowheads. The vertebae are numbered. CC, central canal; MF, Mauthner fibers. Scale bars in panels A and B=0.1 mm. Scale bar in panel C=20 μm.

the cross plane of the spinal cord.

In the sagittal plane of the spinal cord, the somata of all labeled motoneurons were distributed from spinal segment 25 to spinal segment 28 of the spinal cord (Fig. 9). Each motor pool of the anterior caudal-fin muscles stretched over approximately two spinal segments, and each motor pool of the posterior caudal-fin muscles extended over about one spinal segment. For example, the motor pool of the MIR muscle was usually (16/20) located in spinal segment 27 and occasionally (4/20) distributed from spinal segment 27 to the anterior one-third of spinal segment 28. The motoneurons were not evenly distributed in a single motor pool but tended to be clustered (Fig. 5A).

The position of each motor pool was partially overlapping in the spinal cord (Fig. 9). The motor pools of the anterior caudal-fin muscles were located from spinal segment 25 to spinal segment 27, while those of the posterior caudal-fin muscles were distributed from spinal segment 26 to spinal segment 28; thus, the center of the motor pools of the anterior caudal-fin muscles was located more cranially than that of the posterior caudal-fin mus-

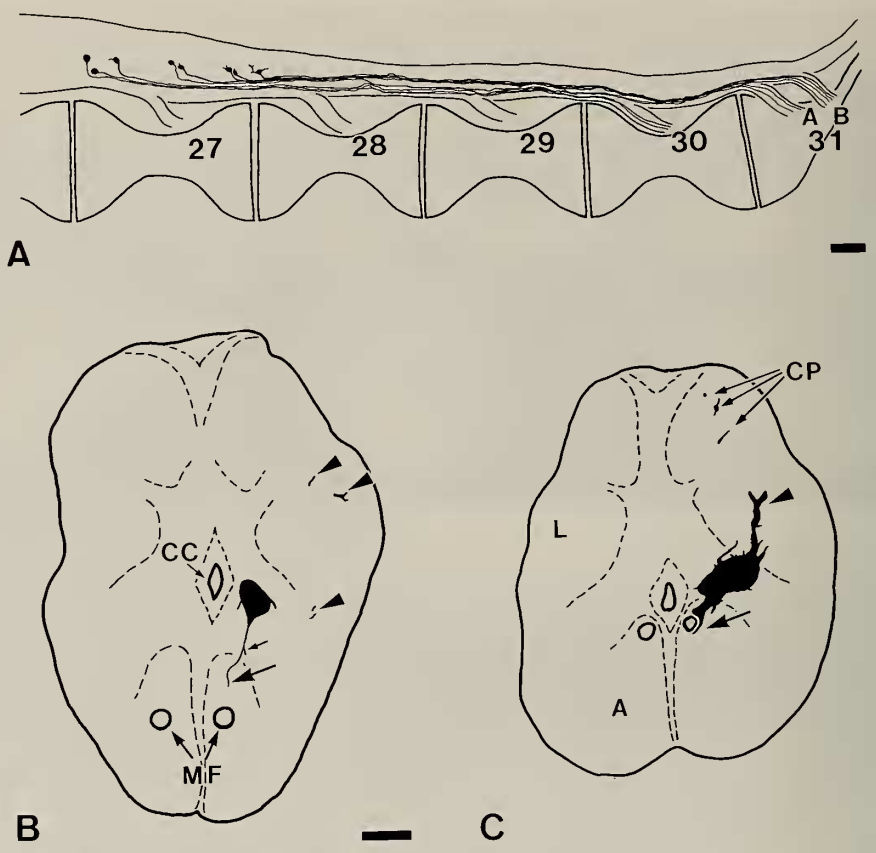


FIG. 6. Drawing of HRP-labeled motoneurons in the adult spinal cords, after applying HRP to the MIR muscle (A and B) and to the HLM (C). In panel A, the left lateral view of the whole mount specimen is shown. The spinal nerves (ventral roots) are numbered. In panels B and C, the typical "small" (B) and "large" (C) motoneurons in cross sections of the spinal cords are shown. In panels B and C, the axons (large arrows), the dendrites (arrowheads), the axon collateral (small arrow), the central processes of the spinal ganglion cells (CP), the anterior funiculus (A), and the lateral funiculus (L) are indicated. CC, central canal; MF, Mauthner fibers. Scale bar in panel A=0.1 mm. Scale bar in panel B=20 μm, valid for panels B and C.

cles. The number of motoneurons in each motor pool filled from a single caudal-fin muscle varied from 1 to 30 (Fig. 9).

Innervation of Caudal-Fin Muscles

By retograde labeling with HRP (Fig. 6A) or DiI (data not shown), the motor axons that project to each caudal muscle were revealed. Thus, the spinal nerves that contribute to the innervation of each caudal-fin muscle were identified (Fig. 10). The DDF was innervated by the rami dorsales from the ventral roots of spinal nerves 28, 29, 30, and 31 (mainly 30 and only slightly 31). The HLM was served by the rami ventrales from the ventral roots of spinal nerves 28, 29, and 30 (mainly 30). The DVF received the rami ventrales of the ventral roots of spinal nerves 27, 28, and 29 (mainly 28 and 29). All interradial muscles were supplied by the rami ventrales of the ventral roots of spinal nerves 30 and 31 (mainly 31).

Muscle Nerve to the MIR Muscle

The peripheral branching pattern of the spinal nerves was examined in detail in the caudal fin (Figs. 11A and 11B). A characteristic pattern, the entire shape of which is like that of a fan-shaped

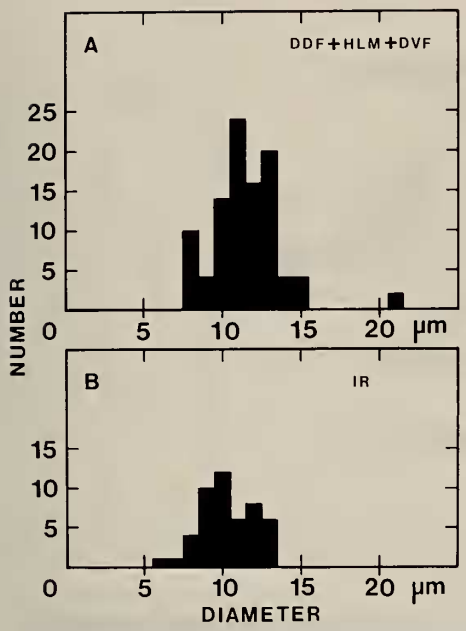


FIG. 7. Size distribution of labeled somata of the motoneurons in adult spinal cords, after applying HRP to the anterior caudal-fin muscles, DDF+HLM+DVF (A) and the posterior caudal-fin muscles, IR (B). All measurements were made on the drawings of the HRP-labeled motoneurons in celloidin sections and were not corrected for shrinkage. The average diameter of an individual cell was calculated as the average of the diameter across the shortest and longest axis of the soma. The ordinate indicates the number of motoneurons. The abscissa indicates the average diameter.

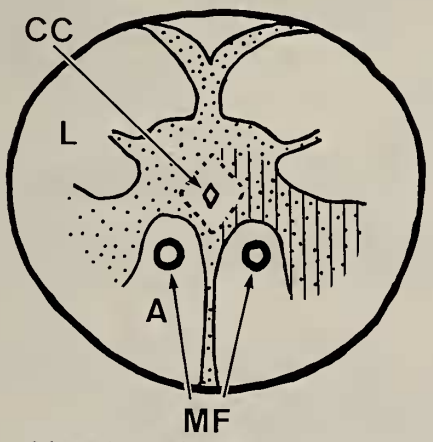


FIG. 8. Schematic drawing of a cross section of the caudal spinal cord, showing the motor column (shaded area) at the right side. The gray matter (dotted area), central canal (CC) surrounded by ependymal cells, and Mauthner fibers (MF) are also shown. A, anterior funiculus; L, lateral funiculus.

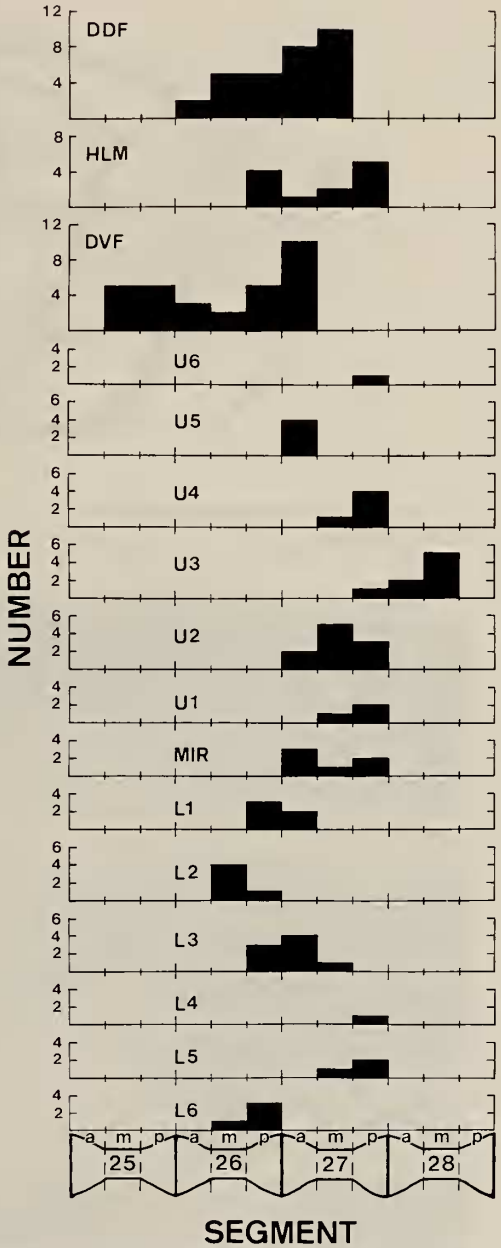


FIG. 9. Histograms showing the distribution pattern of labeled motoneurons along the cranio-caudal axis of the caudal spinal cord, after applying HRP to each caudal-fin muscle of adult fish. A typical result is shown for each muscle. The ordinate indicates the number of labeled motoneurons. The abscissa indicates the spinal segments, each of which is divided into three equal parts; anterior (a), middle (m), and posterior (p). L1–6, lower interradial muscles; U1–6, upper interradial muscles. For other abbreviations, see Figure 3.

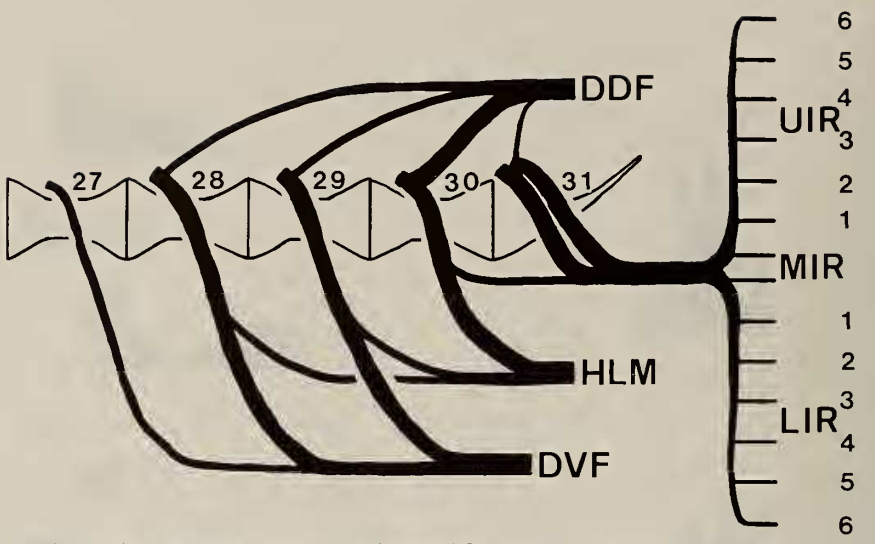
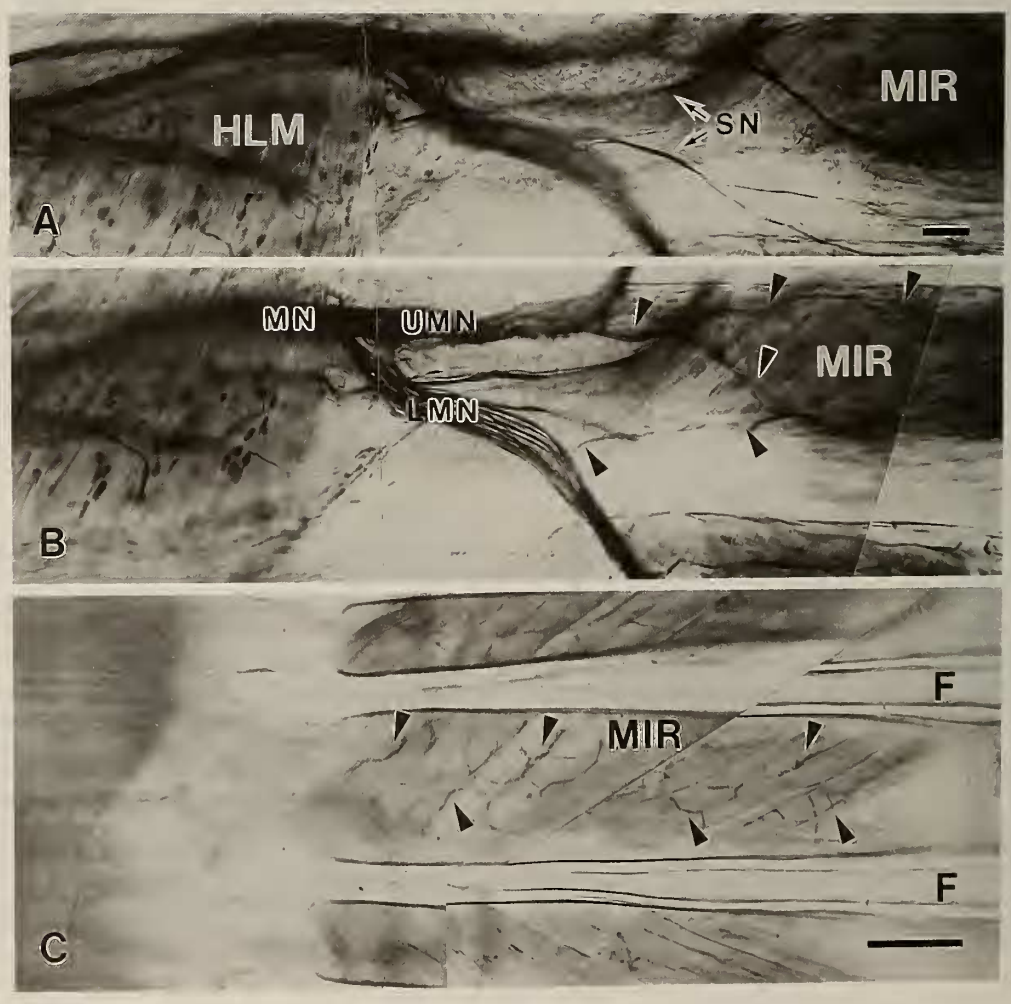


FIG. 10. Schematic drawing of muscle nerves to each caudal-fin muscle of the adult fish. The spinal nerves (ventral roots) are numbered. The main innervating nerves are indicated by thick lines. For abbreviations of muscles, see Figure 3.



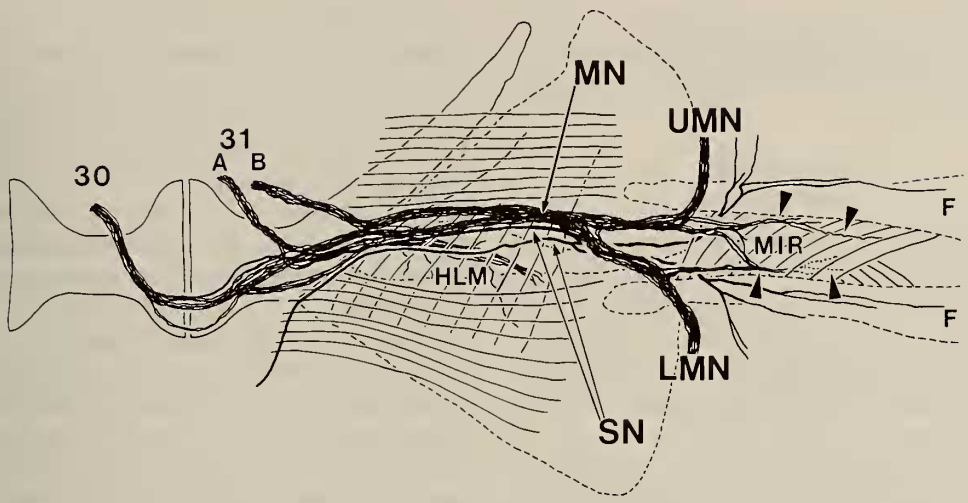


FIG. 12. Drawing of the caudal-fin-motor nerve (MN) and the caudal-fin-sensory nerve (SN) in the left lateral view of the tail region of a young fish. The spinal nerves (rami ventrales) are numbered. The muscle nerve branches in the MIR muscle are indicated by arrowheads. For abbreviations, see Figure 11. Scale bar=0.1 mm.

tree, was found in this region (Figs. 10 and 12): The rami ventrales of the ventral roots of several caudalmost spinal nerves ran ventro-caudally to form a nerve plaxus at the ventral edge of the vertebral column. From the nerve plexus, a thick nerve bundle (caudal-fin-motor nerve) projected caudad. The caudal-fin-motor nerve ran underneath the dorsal part of the HLM toward a space between two central fin rays.

The caudal-fin-motor nerve bifurcated dorsally (upper branch of the caudal-fin-motor nerve) and ventrally (lower branch of the caudal-fin-motor nerve) at the posterior border of the HLM. The two nerve branches ran along the posterior ends of the hypural plates. From these branches, nerve twigs projected and entered the interradial muscles to form the neuromuscular junctions (Fig. 11C).

On the other hand, one or two tightly packed nerve bundles (SN in Figs. 11A and 12) also projected from the nerve plexus. The nerve bundles took a course similar to that of the caudal-fin-motor nerve. However, the distal nerve branches did not stay in the region of the interradial muscles, but extended further along the fin rays to the caudal end of the caudal fin. The nerve bundles were named the caudal-fin-sensory nerve, because putative sensory axons in the caudal fin should be contained in the bundles.

Thus, the courses of the muscle nerves to the interradial muscles were revealed entirely. For example, the muscle nerve to the MIR muscle started from the motoneurons in spinal segment 27 of the spinal cord (Fig. 9), extended caudad in the spinal cord, emerged from the spinal cord *via* the rami ventrales of the ventral roots of the spinal nerves 30, 31A, and 31B, ran further ventro-

FIG. 11. Double staining of muscles and nerves in the caudal fin of a young adult fish (A and B) and AChR staining of interradial muscles in an adult fish (C). The muscles and nerves are immunochemically stained using anti-troponin T antibody and anti-neurofilament protein antibodies, respectively. The AChR is stained immunochemically using erabutoxin b and its antibody. The left lateral views of the whole mount specimens are shown. In panels A and B, the photographs were taken in slightly different fields at different focus planes. The neuromuscular junctions in the MIR muscle (MIR) are observed along the courses of the muscle nerve branches in the MIR muscle (arrowheads). F, central fin rays; LMN, lower branch of the caudal-fin-motor nerve; MN, caudal-fin-motor nerve; SN, caudal-fin-sensory nerve; UMN, upper branch of the caudal-fin-motor nerve. Scale bar in panel A=20 μm, valid for panels A and B. Scale bar in panel C=0.1 mm.

caudally for a long distance (about 600 micra), and finally entered the MIR muscle *via* nerve branches of the upper and lower caudal-fin-motor nerves (Fig. 12).

DISCUSSION

The present study has provided for the first time an anatomical description of the innervation of the caudal-finmuscles of the adult medaka. We found that there was a characteristic pattern of peripheral nerves in the caudal fin and that the motoneurons were organized somatotopically along the cranio-caudal axis of the caudal spinal cord.

The caudalmost musculature in the medaka are derived ontogenetically from several caudalmost myomeres [10]. Also, from phylogenetic studies, it has been pointed out that the caudal-fin muscles of fish are somitic in origin, being derived from caudal myomeres [23]. It might be reasonable to assume, accordingly, that the caudal motoneurons of the medaka are essentially similar to those found in the spinal motoneurons innervating the trunk segmental muscles of the teleost fish.

However, as discussed below, the present results indicate that not only similarities but also differences are present between the caudal motoneurons and the trunk motoneurons.

Innervation and Axonal Pattern

The dorsal caudal-fin muscle, the DDF, is obviously a caudalmost component of the epaxial musculature of the trunk muscle system, while the ventral caudal-fin muscles, the DVF and HLM, are caudalmost components of the hypaxial musculature (Fig. 3). The interradial muscles are the hypaxial muscles as well, because they differentiate in the hypochordal region of the developing caudal fin [2]. Only DDF is innervated by the rami dorsales, while the other muscles are served by the rami ventrales of the ventral roots (Fig. 10). These observations are consistent with studies of trunk segmental muscles in the teleost fish; the studies indicate that the ramus dorsalis and ramus ventralis innervate the epaxial musculature and hypaxial musculature, respectively (see ref. [24] for a review; see also Fig. 2).

In the present study, a motor axon ran longitudinally and caudally for a long distance in the caudal spinal cord, bypassing several ventral roots before entering a single ventral root (Fig. 6A). This axonal pattern in the caudal spinal cord differs from those reported in the trunk spinal cords of the teleost fish. In the trunk spinal cord of the adult goldfish, Fetcho [25] showed that most motor axons ran caudally in the spinal cord and entered the nearest single ventral root, although some motor axons bypassed one root before exiting from the cord. In the rostral trunk region of a larval teleost fish (zebrafish), Myers [26] reported that a single axon of each motoneuron entered the nearest single ventral root, and the motor axons within the given ventral root were all derived from motoneuron somata within a single spinal segment.

Motoneuron Types

The morphology of the trunk motoneurons has been studied in detail in the adult teleost fish using modern techniques ([25], [27], [28]; see ref. [24] for a review). All authors agree that there are two groups of motoneurons; small motoneurons that locate in the ventro-lateral portion of the motor column and larger motoneurons that occupy the dorso-medial part of the motor column.

Raamsdonk *et al.* [27] and Fetcho [25] showed that the differences in the location and morphology of the two types of motoneurons were correlated with differences in the functionally different muscle fiber types they innervate. These investigators reported that "red motoneurons" which innervate the superficial slow (red) muscles are small in their soma size and located in the ventro-lateral part of the motor column; at least some of the "white motoneurons" which innervate the deep fast (white) muscle, were larger and lay near the central canal.

Several authors proposed that the two classes of motoneurons also had different developmental histories [25, 28]. According to these authors, the small motoneurons differentiate later than the larger motoneurons; the small motoneurons and the larger ones were referred to as "secondary motoneurons" and "primary motoneurons", respectively.

In accordance with the above results reported by

other investigators, two classes of soma size were found in the spinal motoneurons innervating the trunk segmental muscles of adult medaka (Ishikawa, unpublished observations): The small motoneurons were about 8–16 micra, while the larger ones were about 20–30 micra in diameter. In the present study too, we observed that there were two populations of soma size in the HRP-labeled motoneurons (the "small motoneurons" and the "large motoneurons"), although there was not a very distinct dichotomy of motoneuronal types (Figs. 6B, 6C and 7).

However, no "large motoneurons" were found in the motor pools of the posterior caudal-fin muscles; only the "small motoneurons" were present. The "small motoneurons" were located over all areas of the motor column including the medial area near the central canal of the spinal cord. Moreover, the "large motoneurons" in the present study were distributed in the lateral area of the cross plane of the motor column (Fig. 6C). Hence, in the motoneurons of the caudal spinal cord of the medaka, it seems inappropriate to apply simply the designation of "white and red" types or "primary and secondary" types of motoneurons which have been reported for the trunk motoneurons.

The "large and small" motoneurons in the present study may reflect the time sequence of development of motoneurons. It is generally accepted that large neurons are produced before small ones in any part of the nervous system [29, 30]. The caudal-fin muscles differentiates later than the trunk segmental muscles [2]. In particular, the posterior caudal-fin muscles develop much later than the anterior caudal-fin muscles. If the motor pools develop in the same sequence as the groups of muscles, the posterior caudal-fin muscles would be innervated by the "small motoneurons". This hypothesis can explain the fact that only the "small motoneurons" are present in the motor pools of the posterior caudal-fin muscles.

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